DATA EVALUATION RECORD
LGC-30473 (Ethaboxam)
PC Code: 090205
TXR#: 0056241
MRID#: 48535644

Study Type: 90-Day Oral Toxicity - Rat;
OPPTS 870.3100

Prepared for

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Disclaimer

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TXR#: 0056241

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity (dietary) - Rat;
OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 090205 **DP BARCODE: D399440**

TEST MATERIAL (PURITY): LGC-30473 (Ethaboxam) (99.2%)

SYNONYMS: Not provided

CITATION: Gardner, T. and P. Higgs (2003) LGC-30473: Toxicity to rats by dietary administration for 13 weeks, Amended report. Huntingdon Life Sciences (Cambridgeshire, England). Laboratory Project ID: LKY 26/963670, July 30, 2003. MRID 48535644. Unpublished.

SPONSOR: Valent U.S.A. Corporation

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 48535644) LGC-30473 (Ethaboxam, 99.2% a.i., Batch # 6-1) was administered to Crl: CD BR rats (10/sex/dose) in the diet at dose levels of 0, 200, 650, or 2000 ppm (equivalent to 0, 16.3, 49.7, or 154 mg/kg bw/day in males; 0, 17.9, 58.0, or 164 mg/kg bw/day in females).

There were no treatment-related effects on mortality. At 200 ppm, there were a few statistically significant effects on hematology and clinical chemistry parameters; in the absence of correlating pathology, the toxicological significance of these findings is questionable. At 650 ppm, treatment-related effects were observed in the male reproductive organs. These included decreased mean absolute epididymides weight with correlating histopathology (increased incidence of abnormal spermatogenic cells in ducts), and an increased incidence of abnormal spermatids in occasional tubules of the testes. Decreased body weight gain and increased liver weights were seen at 650 ppm; however, there were no effects on absolute body weight and no corresponding liver histopathology. A few statistically significant changes in hematology and clinical chemistry parameters, of questionable toxicological significance, were also noted in both sexes at 650 ppm. At 2000ppm, decreased mean absolute body weight in both sexes (up to 21%) , a statistically significant decreased in mean body weight gain in both sexes (Weeks 0-13), decreased total food consumption in males, increased mean adjusted liver weights with a correlating increase in trace centrilobular hepatocyte hypertrophy in both sexes, an increased incidence of fine vacuolation of the zona glomerulosa in the adrenals in females, decreased mean

absolute or adjusted testes weight with correlating severe testicular atrophy (all animals), increased incidence of trace-minimal interstitial cell hyperplasia of the testes, decreased mean absolute prostate weight, and decreased mean absolute epididymides weight with a correlating absence of spermatozoa (all animals) and increased incidence of abnormal spermatogenic cells in occasional ducts. Changes in hematological and clinical chemistry parameters also were found in both sexes at 2000 ppm, some of which (e.g., increased alkaline phosphatase and cholesterol levels in both sexes) may have been related to altered hepatic function. An increased incidence of non-specific hair loss also was observed in females at 2000 ppm.

The LOAEL is 650 ppm (equivalent to 49.7 and 58.0 mg/kg bw/day in males and females, respectively), based on decreased mean absolute epididymides weight in males with correlating histopathology, increased incidence of abnormal spermatids in occasional tubules of the testes in males, and lung effects (increased lung weights, congestion, and alveolar septal congestion and focal alveolar hemorrhage). The NOAEL is 200 ppm (equivalent to 16.3 and 17.9 mg/kg bw/day in males and females, respectively).

This 90-day oral toxicity study in the rat is **acceptable/guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rats.

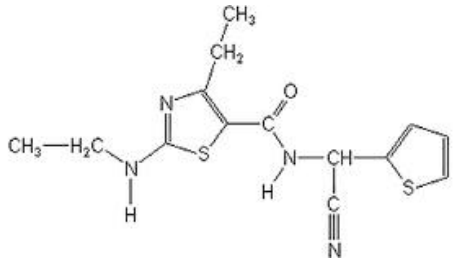
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: LGC-30473 (Ethaboxam)

Description: Creamy-white powder
Batch #: 6-1
Purity: 99.2 % a.i.
Compound stability: No expiration date provided; stored at 4°C in the dark
CAS # of TGAI: 162650-77-3
Structure:



2. Vehicle: The vehicle was the basal diet.

3. Test animals:

Species:	Rat
Strain:	Crl: CD BR
Age/weight at study initiation:	Approx. 6 weeks; 168-201 grams (males); 145-178 grams (females)
Source:	Charles River UK Ltd., Margate, Kent, England
Housing:	5 rats of one sex/cage; suspended cages with wire mesh floors
Diet:	Powdered SDS Rat and Mouse No. 1 Maintenance diet, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 18-23°C
	Humidity: 44-66%
	Air changes: Not provided
	Photoperiod: 12 hrs dark/ hrs light
Acclimation period:	2 weeks

B. STUDY DESIGN:

1. In life dates: Start: August 28, 1996 (initiation of treatment); End: November 27-28, 1996 (terminal sacrifice)

2. Animal assignment: Animals were assigned randomly, stratified by body weight so that the cage means were approximately equal, to the test groups noted in Table 1.

TABLE 1. Study design [a]

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)	# Males	# Females
Control	0	0	10	10
Low	200	16.3 (males) 17.9 (females)	10	10
Mid	650	49.7 (males) 58.0 (females)	10	10

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)	# Males	# Females
High	2000	154 (males) 164 (females)	10	10

[a] Data obtained from page 14 (unnumbered table) and page 39 (Table 5) in the study report.

3. **Dose selection rationale:** The dose levels were selected based on the results from a preliminary 4-week dietary study in rats (Huntingdon Life Sciences Report No. LKY 51/962291), in which dose levels of 3000 ppm and above produced severe adverse effects in both sexes (*Note: specific effects not stated*) and 500 ppm produced no apparent effect. The dose sequence used in the current study (0, 200, 650, 2000 ppm) was considered appropriate to further evaluate potential no effect and high dose levels.

4. **Diet preparation and analysis:** Dietary formulations were prepared weekly. A pre-mix was prepared by grinding the test substance into the basal diet (SDS Rat and Mouse No. 1 Maintenance Diet), then mixing in a Turbula mixer for a minimum of 5 minutes. The dietary concentrations were prepared by dilution of the pre-mix with basal diet, then mixing in a Turbula mixer for a minimum of 5 minutes. The temperature at which the dietary formulations were stored was not provided. The report states that homogeneity and stability were tested prior to the current study on trial diets containing 100, 1000, or 50000 ppm of the test substance. These concentrations bracket the concentrations used in the current study. During the current study, samples of dietary formulations prepared for Weeks 1 and Week 11 were analyzed for acceptability of concentration. The report states that although samples also were taken from formulations prepared for Week 13, they were not analyzed because satisfactory results were obtained for Week 11.

Results:

Homogeneity analysis: The report states that the results of the homogeneity analysis were presented in the preliminary study report (Huntingdon Life Sciences Report No. LKY 51/962291). The results were not summarized in the current report.

Stability analysis: The report states that the results of the stability analysis were presented in the preliminary study report (Huntingdon Life Sciences Report No. LKY 51/962291). The results of the stability analysis were not summarized in the current report; however, the current report states (page12) that the formulations are stable for 22 days at room temperature.

Concentration analysis: The mean concentrations of the samples collected from the 200, 650, and 2000 ppm diets during Week 1 were 198, 634, and 1940 ppm, respectively. The mean concentrations of the samples collected during Week 11were 199, 636, and 1950 ppm, respectively. The test substance was not detected in samples from the untreated basal diet (below the limit of detection of 7.5 ppm). The relative mean errors of the dietary formulations (deviation from nominal concentration) ranged from –0.5% to –3.0%.

The analytical data indicated that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: The following sequence of statistical tests was used for body weight gain, food and water consumption, hematology, biochemistry, urinalysis, and organ weight data. Body weight data were evaluated based only on mean body weight gain over the entire treatment period. Food and water consumption data were analyzed on a cage basis. It is unclear whether mean weekly food consumption and food efficiency data were analyzed for statistical significance, as no statistical results were reported. Total food consumption data for the study period (Weeks 1-13) were statistically evaluated.

Bartlett's test was first applied to test for heterogeneity of variance between the treatment groups. If heterogeneous at the 1% level, a logarithmic transformation was tried to determine if a more stable variance structure could be obtained. If no significant heterogeneity or a satisfactory transformation was found, a one-way analysis of variance (ANOVA) was performed. If the heterogeneity of variance was significant and could not be removed by transformation, an analysis of rank (Kruskal-Wallis) was used. Analyses of variance were followed by Williams' test for a dose-related response. The Kruskal-Wallis analyses were followed by the nonparametric equivalent of this test (Shirley's test).

For organ weight data, analysis of covariance was performed instead of the analysis of variance in the above sequence, using terminal body weight as the covariate when the within group relationship between organ weight and body weight was significant at the 10% level.

Fisher's Exact test was used to analyze the microscopic pathology data. It is not clear whether the macroscopic pathology data were evaluated statistically, as no statistical results were reported.

The Reviewer considers the analyses used to be appropriate.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were observed twice daily on weekdays and once daily on weekends for mortality and moribundity. For at least the first 5 weeks of treatment, animals were observed once daily on weekdays for signs of toxicity or behavioral changes; for the remainder of the study, they were observed once weekly. In the absence of additional information, the Reviewer assumes these observations were performed cageside.

1b. Clinical examinations: No information was provided that indicated that detailed clinical observations, other than palpation, were performed outside of the cage. Animals were palpated once daily on weekdays for the first 5 weeks of treatment. For the remainder of the study, they were palpated once weekly.

1c. Neurological evaluations: Neurological evaluations were not performed.

2. Body weight: Animals were weighed at the time of randomization, on the day treatment was initiated, and once weekly thereafter.

3. **Food/water consumption and compound intake:** Food consumption for each cage was determined weekly and the mean weekly diet consumption was calculated as g food/animal/week. Total food consumption data over the treatment period (Weeks 1-13) were summarized as g/rat. Food efficiency was calculated weekly and as a total over the treatment period as a ratio (food consumed/body weight gain). Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption and body weight gain data.

   Water consumption was monitored daily throughout the study by visual observation. During Week 12, water consumption was measured daily by weight. An explanation was not provided as to why water consumption was measured quantitatively only during Week 12.

4. **Ophthalmoscopic examination:** Eyes of all animals were examined at the time of randomization. The eyes of animals in the control and 2000 ppm groups were examined once during Week 12.

5. **Hematology and clinical chemistry:** During Week 13, blood was collected from all animals (fasted overnight) for hematology and clinical chemistry parameters. Blood was withdrawn from the orbital sinus while the animals were under light ether anesthesia. The CHECKED (X) parameters were examined.

   a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*	X	Cell morphology
X	Activated partial thromboplastin time (APTT)		
	Clotting time		
X	Prothrombin time (PT)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol* (enzymatic assay)
X	Potassium*	X	Globulins [a]
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes e.g., *)		Total bilirubin
X	Alkaline phosphatase (ALK/also AP)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT, GPT)*		
X	Aspartate aminotransferase (AST/also SGOT, GOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

[a] The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration.

6. **Urinalysis:** Urine was collected overnight from all animals during Week 13; animals were fasted and drinking water was removed overnight. The CHECKED (X) parameters were examined.

	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin (bile pigments)
X	pH*	X	Blood/blood cells* (heme pigments)
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen
		X	Total reducing substances (TRS)

* Optional, suggested for 90-day oral rodent studies based on Guideline 870.3100.

7. **Sacrifice and pathology**: All animals were sacrificed on schedule by carbon dioxide asphyxiation and subjected to a detailed gross pathological examination. All superficial tissues were examined visually and by palpation. The cranial roof was removed for observation of the brain, pituitary gland, and cranial nerves. A ventral mid-line incision was made, the skin reflected, and the thoracic, abdominal and pelvic cavity tissues examined. In addition to external examination, the urinary bladder was examined by palpation, and the stomach, cecum and kidneys were incised. The liver was sectioned at intervals of a few millimeters. The CHECKED (X) tissues were collected and fixed in 10% buffered formalin, with the exception of the testes and epididymides, which were fixed in Bouin's solution, and the eyes, which were fixed in Davidson's fixative. The (XX) organs, in addition, were weighed. The tissues listed were examined histologically for animals in the control and high dose group sacrificed after 13 weeks; macroscopically abnormal tissues were examined for animals in all dose groups. In addition, the following tissues, which were found to have treatment-related effects at the 2000 ppm level, were examined for the lower dose groups: liver and adrenals in both sexes, and the testes and epididymides in males.

X	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
	Gall bladder (not rat)*
	Bile duct (rat)
X	Pancreas*

X	RESPIRATORY
X	Trachea*
XX	Lung*
X	Nose* [a]
X	Pharynx*
X	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta*
XX	Heart*+
X	Bone marrow* (sternum)
X	Lymph nodes* (cervical, mesenteric)
XX	Spleen*+
X	Thymus*+

X	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides*+
XX	Prostate*
XX	Seminal vesicles*
XX	Ovaries*+
XX	Uterus*+
X	Mammary gland*
X	Vagina

X	NEUROLOGIC
XX	Brain*+ (medullary, cerebellar, cerebral sections fixed)
X	Peripheral nerve* (sciatic)
X	Spinal cord (3 levels)* [b]
XX	Pituitary*
X	Eyes (optic nerve)*

X	GLANDULAR
XX	Adrenal gland*+
X	Lacrimal gland [a]
X	Parathyroid*
XX	Thyroid*
X	Harderian gland

X	OTHER
X	Bone (sternum, femur)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*
X	Head

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
[a] Included with head.
[b] from cervical level

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** An increased incidence of non-specific hair loss was seen in females in the 2000 ppm group, compared with no incidences in the control group. Hair loss was first noted during Week 1 in 2/10 animals, and increased to 7/10 animals from Weeks 4-13. Yellow stained tray paper was reported for all treatment group cages, first appearing during Week 1 for the 650 and 2000 ppm groups and during Week 3 for the 200 ppm group. The yellow staining was attributed by the Investigators to urinary excretion of the parent compound and/or its metabolites.

2. **Mortality:** There were no unscheduled deaths during the course of the study.

3. **Neurological evaluations:** Neurological evaluations were not performed.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weight gain over the 13-week study period was significantly decreased for males in the 650 and 2000 ppm groups, and for females in the 2000 ppm group. Mean body weights in the 2000 ppm group were much lower than controls in males (up to 21%) and females (up to 16%); mean body weights were not statistically analyzed. Mean body weight gain for males in the 200 ppm group and females in

the 200 and 650 ppm groups was equal to or greater than that of the control. Selected body weight and body weight gain data are presented in Table 2.

TABLE 2. Average body weights and body weight gains during 13 weeks of treatment [a]

Conc. of LGC-30473 in diet (ppm)	Mean body weight (g±SD [b])				Mean total weight gain (Weeks 0-13)	
	Week 0	Week 1	Week 7	Week 13	g ±SD	% of control
Males (n=10)						
0 (control)	186±8.50	244±11.8	447±24.2	502±35.0	316.4±32.5	-
200	189±7.47	245±11.0	457±27.7	511±34,9	322.4±36.9	102
650	188±6.96	237±9.88	422±31.2	468±45.2	279.7*±45.7	88
2000	179±7.71	210±10.6 (↓14%)	360±23.9 (↓9%)	397±34.9 (↓21%)	217.7**±32.7	69
Females (n=10)						
0 (control)	158±7.12	183±7.43	266±14.9	282±24.5	123.5±21.8	-
200	161±8.00	187±11.8	276±18.8	294±20.5	132.3±17.8	107
650	162±9.46	183±13.6	271±14.8	285±22.9	123.3±16.7	100
2000	156±7.04	164±7.03 (↓10%)	227±11.1 (↓15%)	237±10.9 (↓16%)	80.7**±9.5	65

[a] Data obtained from page 24 (unnumbered table) and page 36 (Table 2) in the study report.
[b] The standard deviations were calculated by the Reviewer from data on pages 62-65 (Appendix 1) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Total food consumption (g/rat) was reduced over the course of the study (Weeks 1-13) for males in the 650 ppm and 2000 ppm groups (8% and 18%, respectively) and for females in the 2000 ppm group (22%), when compared with the control. The total intake reached statistical significance for males in the 2000 ppm group. The results for food consumption are consistent with the reduced mean body weight gains observed in these groups. The data for total food consumption are summarized in Table 3.

TABLE 3. Total food consumption during 13 weeks of treatment [a]

Conc. of LGC-30473 in diet (ppm)	Total food consumption (Weeks 1-13) (g/rat±SD)	% of Control
Males		
0 (control)	2730±41	-
200	2884±142	106
650	2517±121	92
2000	2229*±96	82
Females		
0 (control)	2020±86	-
200	2045±242	101
650	2003±178	99
2000	1572±68	78

[a] Data obtained from page 25 (unnumbered table) in the study report.
* Statistically different ($p < 0.05$) from the control.

2. **Compound consumption:** The time-weighted average test substance consumption values are summarized above in Table 1.

3. **Food efficiency:** Food efficiency over the 13 weeks of treatment, as indicated by a higher food conversion ratio (food consumption/body weight gain) was notable for both sexes in the 2000 ppm group (approximately 19% higher than the control for both sexes). The food conversion ratios for the 13-week treatment period are summarized in Table 4.

TABLE 4. Food conversion ratios for the 13 weeks of treatment [a]

Conc. of LGC-30473 in diet (ppm)	Food conversion ratio (Weeks 1-13)
Males	
0 (control)	8.6
200	8.9
650	9.0
2000	10.2
Females	
0 (control)	16.4
200	15.5
650	16.2
2000	19.5

[a] Data obtained from page 25 (unnumbered table) in the study report.

4. **Water consumption:** Water consumption was evaluated by visual observation, with the exception of Week 12, when it was quantitatively assessed on a daily basis. Water consumption was reported to be highly variable during the course of the study. A significant decrease in mean water consumption was seen in females in all treatment groups during Week 12. Males in the 2000 ppm group showed a decrease (17%) similar to that of females in the 200 and 650 ppm groups (15-16%) when compared with the control; however, the value for males did not reach statistical significance. Water consumption data for Week 12 are summarized in Table

TABLE 5. Water consumption - Week 12 [a]

Conc. of LGC-30473 in diet (ppm)	Total water consumption Week 12 (g/rat±SD)	% of Control
Males		
0 (control)	242±17.1	-
200	279±0.1	115
650	227±31.4	94
2000	200±15.8	83
Females		
0 (control)	224±19.5	-
200	191*±11.5	85
650	188*±0.2	84
2000	139**±3.2	62

[a] Data obtained from page 26 (unnumbered table) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

D. OPHTHALMOSCOPIC EXAMINATION: No abnormalities were reported either pre-dose or in Week 12.

E. BLOOD ANALYSES:

1. Hematology: Several changes in hematological parameters were noted in blood samples collected in Week 13. The mean activated partial thromboplastin time (APTT) was significantly increased in females in the 2000 ppm group; mean platelet counts were noticeably reduced in females in the 650 and 2000 ppm groups (16% and 18%, respectively), but the differences did not reach statistical significance. Mean prothrombin time was significantly decreased in females in the 2000 ppm group when compared with the control, although the reduction was small (4%) and a clear dose-response relationship was not found. A dose-related decrease in mean eosinophil count was found in males, reaching statistical significance in the 2000 ppm group. A statistically significant decrease in mean basophil count was observed in males at all treatment levels, although the dose-response was not consistent (43%, 57%, and 29% at 200, 650, and 2000 ppm, respectively). A dose-related decrease in mean neutrophil count was seen in males, although the decreases did not reach statistical significance; mean neutrophil counts also were reduced in females (not statistically significant), with no consistent dose-response trend. A statistically significant decrease in mean monocyte count (33%) was found in females in the 2000 ppm group. A small, but statistically significant decrease in mean corpuscular hemoglobin concentration (MCHC) was observed in females in the 650 and 2000 ppm groups (2% and 8%, respectively). Because the decreases in MCHC were very small and there were no other significant differences in hematological parameters for females in these groups, including the number of erythrocytes, that would suggest a treatment-related effect on red blood cell production, it is unlikely that the effects seen in these groups were treatment-related. No microscopic and/or macroscopic treatment-related changes were reported for lymphoid organs (spleen, thymus, lymph nodes) or bone marrow. Data for selected hematological parameters are summarized in Table 6.

TABLE 6. Selected hematological parameters - Week 13 [a]

Parameter (mean±SD)	Concentration of LGC-30473 in diet (ppm)			
	0 (control)	200	650	2000
Males (n=10)				
MCHC (g/dL)	35.1±0.49	35.1±0.37	35.3±0.50	35.1±0.45
Platelets (x 10^9/L)	1023±118.0	1011±102.2	1066±81.6	990±103.5
Prothrombin time (s)	16.7±0.54	17.3±0.53	17.0±0.73	16.8±0.78
APTT (s)	21.6±1.14	21.7±1.23	21.5±1.67	22.4±1.41
Neutrophils (x 10^9/L)	1.77±0.722	1.62±0.614	1.39±0.294	1.28±0.485
Eosinophils (x 10^9/L)	0.26±0.173	0.23±0.198	0.17±0.112	0.14*±0.055
Basophils (x 10^9/L)	0.07±0.024	0.04**±0.013	0.03**±0.013	0.05**±0.011
Monocytes (x 10^9/L)	0.16±0.083	0.13±0.054	0.11±0.038	0.14±0.014
Females (n=10)				
MCHC (g/dL)	36.0±0.59	35.8±0.51	35.2*±0.31	35.7*±0.49
Platelets (x 10^9/L)	1045±164.4	1082±163.2	877±323.0	862±127.3
Prothrombin time (s)	17.0±0.87	16.4±0.36	16.8±0.70	16.3*±0.74
APTT (s)	17.6±0.65	17.4±1.06	17.8±0.93	19.1**±1.14
Neutrophils (x 10^9/L)	1.25±0.596	0.89±0.294	0.88±0.258	0.97±0.268

Eosinophils (x 10^9/L)	0.18±0.047	0.17±0.050	0.15±0.052	0.17±0.063
Basophils (x 10^9/L)	0.02±0.005	0.02±0.016	0.02±0.010	0.01±0.007
Monocytes (x 10^9/L)	0.12±0.039	0.10±0.039	0.11±0.039	0.08*±0.028

[a] Data obtained from pages 41-42 (Table 7) and pages 71-78 (Appendix 5) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Clinical chemistry:** Dose-related increases (18-32%) in mean alkaline phosphate (AP) levels were observed in all female treatment groups, reaching statistical significance in the 2000 ppm group. The mean AP level was increased to a lesser extent in males in the 2000 ppm group (9%) and was not statistically significant. A statistically significant decrease in the mean GPT value (18%) was found in males in the 2000 ppm group, and a significant decrease in the mean GOT value (25%) was found in females in the 2000 ppm group. The mean GPT value in females and mean GOT value in males in the 2000 ppm group were reduced 29% and 12%, respectively; neither were statistically significant.

   Small (2%-9%), but statistically significant decreases in mean calcium levels were found in both sexes in the 650 and 2000 ppm groups. Mean albumin levels were statistically significantly decreased in males in all treatment groups, although the difference from the control was slight (<3.5%), with only a small difference between the treatment groups; the mean albumin level was significantly decreased (9%) in females in the 2000 ppm group. A dose-related, statistically significant decrease (8-13%) in mean phosphorus levels was reported for males in all treatment groups. Mean cholesterol levels were significantly increased (43-49%) in both sexes in the 2000 ppm group. Other statistically significant changes in blood clinical chemistry parameters in the 2000 ppm group were: increased mean globulin in both sexes (5-11%), increased mean urea nitrogen in males (17%), decreased mean glucose in females (12%), and decreased mean potassium in males (8%).

   Some of the changes in clinical chemistry (e.g., increased AP and cholesterol levels in both sexes at 2000 ppm) may be a result of treatment-related effects on the liver. Both sexes in this group had a significantly increased mean adjusted liver weight (Table 9), and an increased incidence of trace centrilobular hepatocyte hypertrophy (Table 11). Data for selected clinical chemistry parameters are summarized in Table 7.

TABLE 7. Selected clinical chemistry parameters – Week 13 [a]

Parameter (mean±SD)	Concentration of LGC-304733 in diet (ppm)			
	0 (control)	200	650	2000
Males (n=10)				
Glucose (mg/dL)	117±12.1	125±11.5	119±10.5	115±12.5
Albumin (g/dL)	2.9±0.13	2.8*±0.17	2.8*±0.11	2.9*±0.12
Globulin (g/dL)	3.8±0.22	3.9±0.22	3.9±0.21	4.0*±0.27
Urea nitrogen (mg/dL)	12±1.3	13±1.4	12±1.1	14*±1.7
AP (mU/mL)	179±30.4	177±33.3	180±15.2	195±35.6
GPT (mU/mL)	33±3.6	31±7.1	29±3.7	27*±5.0
GOT (mU/mL)	58±5.2	63±12.2	57±6.0	51±6.6
Potassium (mEq/L)	3.8±0.0.20	3.7±0.26	3.6±0.14	3.5**±0.21
Calcium (mEq/L)	5.6±0.14	5.5±0.08	5.4*±0.12	5.4**±0.15
Phosphorus (mEq/L)	3.9±0.34	3.6*±0.17	3.5**±0.20	3.4**±0.28
Cholesterol (mg/dL)	71±19.3	80±12.2	83±28.4	106**±18.0
Females (n=10)				
Glucose (mg/dL)	115±16.1	119±9.5	122±11.2	101*±9.8
Albumin (g/dL)	3.4±0.12	3.5±0.34	3.2±0.27	3.1*±0.20

Globulin (g/dL)	3.8±0.23	4.0±0.28	3.9±0.22	4.2**±0.29
Urea nitrogen (mg/dL)	15±1.3	17±2.4	17±2.8	17±2.0
AP (mU/mL)	84±13.4	99±32.7	100±15.8	111**±18.6
GPT (mU/mL)	35±12.5	33±14.2	26±7.2	25±5.1
GOT (mU/mL)	69±21.3	56±15.1	56±8.8	52*±7.2
Potassium (mEq/L)	3.3±0.20	3.3±0.21	3.4±0.29	3.5±0.20
Calcium (mEq/L)	5.7±0.17	5.7±0.16	5.4**±0.21	5.2**±0.14
Phosphorus (mEq/L)	3.0±0.35	2.9±0.31	2.8±0.41	2.8±0.29
Cholesterol (mg/dL)	91±13.3	96±21.2	96±19.5	130**±19.7

[a] Data obtained from pages 43-44 (Table 8) and pages 79-86 (Appendix 6) in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

F. URINALYSIS: A statistically significant decrease (43%) in urine volume was found in males in the 2000 ppm group; urine volume also was notably decreased in males in the 650 ppm group (24%), but the difference did not reach statistical significance. Females in the 650 and 2000 ppm groups showed an increase in urinary protein (12% and 27%, respectively); the differences were not statistically significant. Selected urinalysis data are presented in Table 8.

TABLE 8. Selected urinalysis parameters during Week 13 of treatment [a]

Parameter (mean±SD)	Concentration of LGC-30473 in diet (ppm)			
	0 (control)	200	650	2000
Males (n=10)				
Volume (mL)	8.0±2.43	8.3±2.08	6.1±1.52	4.6**±2.11
Protein (mg/dL)	132±68.4	131±52.3	185±105.2	141±49.3
Females (n=10)				
Volume (mL)	2.5±1.22	4.1±1.50	3.2±1.80	2.3±1.17
Protein (mg/dL)	74±14.3	74±26.6	83±25.2	94±14.4

[a] Data obtained from page 45 (Table 9) and pages 87-94 (Appendix 7) in the study report.
** Statistically different ($p < 0.01$) from the control.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: A statistically significant increase in mean adjusted liver weight was found in females in the 650 and 2000 ppm groups, and in males in the 2000 ppm group. The liver changes in the 2000 ppm group were accompanied by centrilobular hypertrophy in both sexes.(Table 11). A small, statistically significant increase in mean adjusted brain weight (6%) was found in males in the 2000 ppm group; there were no reported corresponding macroscopic or microscopic changes.

For males in the 2000 ppm group, a statistically significant decrease in mean absolute or adjusted weight was noted for the right and left testes, respectively. This finding correlates with severe testicular atrophy, seen in all animals in this group (Tables 10 and 11). The mean absolute epididymides weight was significantly decreased at 650 ppm (right side) and 2000 ppm (left and right sides); the mean left epididymides weight of the 650 ppm group was similar to that of the right side, but did not reach statistical significance. Spermatozoa were absent from the epididymides of all animals in the 2000 ppm group (Table 11). The mean absolute prostate weight also was statistically significantly decreased in males in the 2000 ppm group. The mean absolute seminal vesicle weight was decreased in the 650 and 2000 ppm groups; but the changes did not reach statistical significance, and there were no reported macroscopic or microscopic findings. The mean absolute uterus weight for females in the

2000 ppm group was slightly reduced (4%, not statistically significant); this finding may be related to the decreased uterus size observed in 3/10 animals in this group (Table 10); there were no reported histopathologic correlates.

A slight increase in mean absolute lung weight (not statistically significant) was seen in males in the 650 and 2000 ppm groups (4% and 9%, respectively) and females in all treatment groups (11%, 22%, and 12% at 200, 650, and 2000 ppm, respectively). The increases are likely related to the lung congestion noted in 2-5 animals of each of these groups (Table 10).

There were no other statistically significant changes in mean organ weights, or changes in weight that could be related to macroscopic or microscopic pathological changes. Selected organ weight data are summarized in Table 9.

TABLE 9. Selected absolute and adjusted organ weights (Week 14) [a]

Parameter	Concentration of LGC-30473 in diet (ppm)			
	0 (control)	200	650	2000
Males (n=10)				
Mean terminal body weight (g±SD)	496±34.9	508±36.8	464±45.1	394±37.4
Mean absolute (g±SD) and adjusted [b] (g) organ weights				
Liver				
Absolute	21.1±2.70	22.3±2.56	19.4±3.68	18.8±2.42
Adjusted	19.5	19.9	19.4	22.8*
Lungs				
Absolute	1.78±0.195	1.79±0.209	1.86±0.238	1.94±0.353
Brain				
Absolute	2.02±0.065	2.08±0.120	2.00±0.056	2.04±0.064
Adjusted	1.99	2.04	2.00	2.11*
Prostate				
Absolute	0.940±0.1635	1.077±0.1408	0.979±0.1807	0.787*±0.1028
Seminal vesicle				
Absolute	1.46±0.335	1.50±0.368	1.34±0.333	1.18±0.195
Testes (Left)				
Absolute	1.804±0.1401	1.830±0.1997	1.833±0.1091	0.861±0.2076
Adjusted	1.758	1.767	1.834	0.969**
Testes (Right)				
Absolute	1.808±0.1464	1.810±0.1398	1.849±0.1528	0.803**±0.1070
Epididymides (Left)				
Absolute	0.642±0.0666	0.676±0.0715	0.590±0.0561	0.361**±0.0392
Epididymides (Right)				
Absolute	0.683±0.0741	0.684±0.0587	0.594**±0.0563	0.363**±0.0316
Females (n=10)				
Mean terminal body weight (g±SD)	282±24.3	292±21.8	286±22.5	239±9.0
Mean absolute (g±SD) and adjusted [b] (g) organ weights				
Liver				
Absolute	10.5±1.31	11.7±1.65	12.0±1.23	10.5±1.19
Adjusted	10.2	10.9	11.5**	12.1**
Lungs				
Absolute	1.32±0.157 [c]	1.47±0.175	1.61±0.380	1.48±0.263
Brain				
Absolute	1.89±0.078	1.98±0.051	1.91±0.046	1.85±0.087
Adjusted	1.88	1.95	1.89	1.90
Uterus				

Parameter	Concentration of LGC-30473 in diet (ppm)			
	0 (control)	200	650	2000
Absolute	0.54±0.135	0.65±0.229	0.57±0.101	0.52±0.194

[a] Data obtained from pages 46-49 (Table 10) and pages 95-100 (Appendix 8) in the study report.
[b] Organ weights were adjusted for the body weight as covariate, when necessary.
[c] n=9
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Gross pathology:** All males in the 2000 ppm group had small, blue testes and small epididymides, compared with no findings in the control or other treatment groups. These findings correlate with decreased mean testes and epididymides weights noted in this group (Table 9). A small uterus was noted in 3/10 females in the 2000 ppm group, compared with no findings in the control group; a decreased mean uterus weight (not statistically significant) was found in this group (Table 9). Increased incidences of lung congestion were observed in both sexes in the 650 and 2000 ppm groups. Microscopic findings (Table 11) confirmed increased incidences of lung congestion in these groups; mean absolute lung weights also were increased (Table 9, not statistically significant). Other macroscopic findings were not dose-related. Selected macroscopic findings are summarized in Table 10.

TABLE 10. Selected macroscopic necropsy findings [a]

Organ/finding	Number of findings							
	Concentration of LGC-30473 in diet (ppm)							
	0 (control)	200	650	2000	0 (control)	200	650	2000
	Males (n=10)				Females (n=10)			
Lungs								
Congested	0	0	3	4	0	2	5	5
Testes								
Small	0	0	0	10	-	-	-	-
Blue	0	0	0	10	-	-	-	-
Epididymides								
Small	0	0	0	10	-	-	-	-
Uterus								
Small	-	-	-	-	0	0	0	3

[a] Data obtained from pages 50-51 (Table 11) in the study report.

3. **Microscopic pathology:** A statistically significant increase in incidences of trace centrilobular hepatocyte hypertrophy was observed in males (10/10) and females (8/10) in the 2000 ppm group. These findings correlate with a statistically significant increase in mean adjusted liver weight found in both sexes of this group (Table 9). An increased incidence of fine vacuolation of the zona glomerulosa was seen in males (3/10) and females (8/10) in the 2000 ppm group; the increase was statistically significant only in females. A small increase in mean adjusted adrenals weight was seen in all female treatment groups (Table 9); however, the differences from the control (8-14%) were not statistically significant.

Histopathological effects on male reproductive organs were observed in the 650 and 2000 ppm groups. At 650 ppm, there were statistically significant increases in incidences of abnormal spermatids in occasional testes tubules (4/10) and abnormal spermatogenic cells in ducts of the epididymides (6/10). At 2000 ppm, there were statistically significant increases in incidences of severe testicular atrophy (10/10), trace-minimal interstitial cell hyperplasia in the testes (8/10), absence of spermatozoa in the epididymides (10/10), and abnormal

spermatogenic cells in occasional ducts of the epididymides (8/10). These findings correlate with decreased weights found for the testes (2000 ppm) and epididymides (650 and 2000 ppm) (Table 9) and macroscopically observed decreases in testes and epididymides size (Table 10).

Increases in incidences of alveolar septal congestion and focal alveolar hemorrhage were reported in both sexes of the 650 and 2000 ppm groups; these changes did not reach statistical significance. The findings in the lungs correlate with the macroscopic observation of increased lung congestion (Table 10) and increased absolute lung weights (Table 9, not statistically significant) in these groups. An increased incidence of prominent ultimobranchial cysts was observed in both sexes in the 2000 ppm group (not statistically significant).

Other microscopic changes did not show a dose-response trend and/or were found in only 1-2 animals/group. Selected microscopic findings are summarized in Table 11.

TABLE 11. Selected microscopic necropsy findings [a]

Organ/finding	Number of findings							
	Concentration of LGC-30473 in diet (ppm)							
	0 (control)	200	650	2000	0 (control)	200	650	2000
	Males				Females			
Lungs *(# examined)*	*(10)*	*(1)*	*(3)*	*(10)*	*(10)*	*(2)*	*(5)*	*(10)*
Focal alveolar hemorrhage	2	0	1	4	0	1	1	3
Focal alveolar septal congestion (trace-minimal)	2	0	3	4	3	2	5	9
Diffuse alveolar septal congestion (trace-minimal)	1	0	0	3	0	0	0	0
Liver *(# examined)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*
Centrilobular hepatocyte hypertrophy (trace)	0	0	0	10**	0	0	0	8**
Adrenals *(# examined)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*	*(10)*
Fine vacuolation of zona glomerulosa	0	0	0	3	1	1	0	8**
Thyroid *(# examined)*	*(10)*	*(0)*	*(0)*	*(10)*	*(10)*	*(0)*	*(0)*	*(10)*
Prominent ultimobranchial cysts	1	-	-	6	2	-	-	4
Testes *(# examined)*	*(10)*	*(10)*	*(10)*	*(10)*	-	-	-	-
Severe atrophy	0	0	0	10**	-	-	-	-
Abnormal spermatids in occasional tubules	0	0	4*	0	-	-	-	-
Interstitial cell hyperplasia (trace-minimal)	0	0	0	8**	-	-	-	-
Epididymides *(# examined)*	*(10)*	*(10)*	*(10)*	*(10)*	-	-	-	-
Spermatozoa absent	0	0	0	10**	-	-	-	-
Abnormal spermatogenic cells in occasional ducts	0	0	1	8**	-	-	-	-
Abnormal spermatogenic cells in ducts	0	0	6**	0	-	-	-	-

[a] Data obtained from pages 29-30 (unnumbered tables) and pages 52-61 (Table 12) in the study report.
* Statistically different ($p \leq 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: This study generally showed dosage-related toxic

effects of treatment at 650 and 2000 ppm. The main adverse effect was consistently reduced food intake that resulted in growth retardation. The efficiency of food utilization was also impaired at 2000 ppm, suggesting toxicity rather than unpalatability of the test substance. The prevalence of hair loss among affected females was considered possibly indicative of nutritional imbalances caused by poor food consumption. Treatment-related effects were seen on the testes and epididymides at 650 and 2000 ppm, and on the liver at 2000 ppm. The changes in the liver were considered to represent adaptive, rather than toxic, responses to treatment. Minor physiological changes were observed during the last week of treatment at 650 and 2000 ppm; the findings were considered to be of unknown toxicological importance or unlikely to be of toxicological importance. The changes seen at 200 ppm were few, minimal in degree, and without treatment-related pathological findings.

In the absence of treatment-related pathological findings, 200 ppm was identified as a no-observable-effect level, equivalent to an average intake of 16.3 or 17.9 mg/kg/day for males and females, respectively.

B. REVIEWER COMMENTS:

All treatment levels were associated with yellow coloration of the urine, which was attributed by the Investigators to excretion of considerable amounts of the parent compound and/or metabolites.

200 ppm: A few statistically significant changes, mostly affecting hematological and clinical parameters, were observed in the 200 ppm group. A statistically significant decrease in mean basophil count was observed in males, but the dose-response was inconsistent. A statistically significant decrease in mean serum albumin level was seen in males; the reduction, compared with the control, was very small (3%) and was approximately the same as that seen at higher dose levels. A small reduction (8%) in mean serum phosphorous level also was noted in males. A statistically significant decrease in mean water consumption was measured for females during Week 12 of the study; the reason for the decrease, also observed at the higher dose levels, is uncertain. In the absence of other evidence of potential toxicological effects at this dose level, including changes in mean body weight, or gross and microscopic pathology, the findings at 200 ppm are of questionable toxicological significance.

650 ppm: Signs of toxicity affecting the male reproductive organs were seen in animals in the 650 ppm group. Decreased mean absolute epididymides weight in males with correlating histopathology (increased incidence of abnormal spermatogenic cells in ducts), increased incidence of abnormal spermatids in occasional tubules of the testes, and increased mean adjusted liver weight in females. Also observed were statistically significant changes in hematological parameters (decreased mean basophil count in males, decreased mean MCHC in females), changes in clinical chemistry parameters (decreased mean albumin in males, decreased mean calcium in both sexes, and decreased mean phosphorous in males), and decreased water consumption in females (Week 12); in the absence of related pathological observations, these findings are of questionable toxicological significance.

Increased incidences of alveolar septal congestion and focal alveolar hemorrhage were reported in both sexes at ≥ 650 ppm; the changes did not reach statistical significance. The findings in the lungs correlate with the macroscopic observation of increased lung congestion

and increased absolute lung weights.

2000 ppm: More severe toxicity was evident in the 2000 ppm group, affecting body weight, food consumption, the liver and adrenals, and male reproductive organs. Decreased body weight and statistically significant decreases in mean body weight gain in both sexes (Weeks 0-13), with correlating decreases in mean food consumption (statistically significant in males) and mean food utilization efficiency, increased mean adjusted liver weights in both sexes with correlating histopathology (increase incidence of trace centrilobular hepatocyte hypertrophy), decreased mean absolute or adjusted testes weight in males with correlating macroscopic and microscopic pathology (severe testicular atrophy in all animals, increased trace-minimal interstitial cell hyperplasia), decreased mean absolute prostate weight, decreased mean absolute epididymides weight with correlating macroscopic and microscopic pathology (small epididymides, absence of spermatozoa in all animals, increased incidence of abnormal spermatogenic cells in occasional ducts), and an increased incidence of fine vacuolation of the zona glomerulosa of the adrenals in females.

Other statistically significant changes at 2000 ppm included changes in hematological parameters (decreased mean eosinophil and basophil counts in males, decreased mean monocyte count in females, decreased mean prothrombin time and increased mean APTT values in females, decreased mean MCHC in females), changes in clinical chemistry parameters (decreased mean glucose in females, decreased mean albumin in both sexes, increased mean globulin in both sexes, increased mean urea nitrogen in males, increased mean AP value in females, decreased mean GPT value in males, decreased mean GOT value in females, decreased mean potassium in males, decreased mean calcium in both sexes, decreased mean phosphorous in males, increased mean cholesterol in both sexes), increased mean adjusted brain weight in males, decreased mean water consumption in females, and decreased mean urine volume in males (Week 13). Increased incidences of small uterus and nonspecific hair loss also were observed in females (statistical significance not reported).

Increased incidences of alveolar septal congestion and focal alveolar hemorrhage and increased lung congestion and absolute lung weights were also seen at 2000 ppm. Additionally, increased incidences of prominent ultimobranchial cysts were observed in both sexes in the 2000 ppm group (not statistically significant).

The LOAEL is 650 ppm (equivalent to 49.7 and 58.0 mg/kg bw/day in males and females, respectively), based on decreased mean absolute epididymides weight in males with correlating histopathology, increased incidence of abnormal spermatids in occasional tubules of the testes in males, and lung effects (increased lung weights, congestion, and alveolar septal congestion and focal alveolar hemorrhage). The NOAEL is 200 ppm (equivalent to 16.3 and 17.9 mg/kg bw/day in males and females, respectively).

C. STUDY DEFICIENCIES:

There were no major study deficiencies that would have affected the outcomes of the study. The study may have been deficient in detailed clinical observations. No information was provided that indicated that detailed clinical observations were made outside of the home

cage. In addition, a neurological evaluation (i.e., assessment of motor activity, grip strength, and sensory activity) near the end of the exposure period, recommended by the guidelines, was not performed. However, there were no reported cageside findings that would suggest functional deficits resulting from test substance treatment.

It may be noted that the study was performed in 1996, prior to the publication of the current OPPTS 870.3100 guidelines in 1998.

Information regarding the stability of the test substance (e.g., expiration date) and details of the results of previously conducted dietary formulation stability and homogeneity studies were not provided. This information should be submitted, if not included in other studies submitted to the Agency.